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(Direktor: Prof. Dr. O. Wieland)

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Erschien unter dem Titel „Intracellular Localization of Pyruvate
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Intracellular Localization of Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase in Rat Liver

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The overall activity and distribution of pyruvate carboxylase and of phosphoenolpyruvate carboxykinase in rat liver was studied by applying an improved technique for the fractional extraction of hepatic enzymes. In the rat the mean activity of pyruvate carboxylase amounted to 10 units/g fresh liver (1 unit = 1 μ mole pyruvate carboxylated/min at 30°). In the human liver the corresponding value was about 1 unit/g fresh wt. For phosphoenolpyruvate carboxykinase 2.5 and 10 units/g fresh wt. were measured in rat and human liver, respectively. During fractional extraction only negligible quantities of pyruvate carboxylase appeared in the soluble cytoplasmatic fraction. It is concluded that the bulk of enzyme activity is located within the mitochondria. By digitonin treatment of isolated rat liver mitochondria pyruvate carboxylase could be localized within the matrix space. Phosphoenolpyruvate carboxykinase was found to be located mainly in the soluble cytoplasmatic fraction. Our results strongly support the view that in rat liver gluconeogenesis pyruvate has to enter the mitochondria prior to its carboxylation to oxaloacetate.

From the regulatory point of view the localization of pyruvate carboxylase, a key enzyme for hepatic gluconeogenesis, deserves special interest. Several authors have reported rat liver pyruvate carboxylase to be localised exclusively within the mitochondrion [1–5] while others have suggested an extramitochondrial “soluble” pyruvate carboxylase [6–8]. We have reinvestigated this problem by applying a technique which allows fractional extraction of tissue proteins under highly standardized and therefore reproducible conditions [9,10]. Our results reported herein strongly support the view that rat liver pyruvate carboxylase is localized exclusively within the

mitochondrion. Furthermore, our data suggest that, in the mitochondrion, pyruvate carboxylase is a component of the matrix space.

MATERIALS AND METHODS

Fractional Extraction of Liver

Livers of Wistar II as well as Sprague Dawley rats, fasted for 24–48 hours, were subjected to fractional extraction of extra- and intramitochondrial enzymes according to the method of Pette [9,10].

Fractional Extraction of Mitochondria

An extraction of the cristae space of isolated rat liver mitochondria was achieved by the following procedure. Mitochondria suspended in 0.44 M sucrose medium (0.44 M sucrose, 10 mM triethanolamine buffer, 2 mM EDTA, pH 7.4) were incubated 20 min at 4° with digitonin (2.1 mg digitonin/10 mg of mitochondrial protein). The mitochondria were then sedimented at 9,500 $\times g$ and washed once with fresh sucrose medium. The extraction of the matrix space was then achieved by exposure of the digitonin-treated mitochondria to sonic oscillation and a subsequent extraction in 0.1 M phosphate buffer, pH 7.3.

Enzymes. Adenylate kinase, or ATP: AMP phosphotransferase (EC 2.7.4.3); citrate synthase, or citrate oxaloacetate-lyase (CoA-acetylating) (EC 4.1.3.7); fumarate hydratase, or L-malate hydro-lyase (EC 4.2.1.2); glutamate dehydrogenase (NAD), or L-glutamate: NAD oxidoreductase (deaminating) (EC 1.4.1.3); 3-hydroxyacyl-CoA dehydrogenase, or L-3-hydroxyacyl-CoA: NAD oxidoreductase (EC 1.1.1.35); malate dehydrogenase, or L-malate: NAD oxidoreductase (EC 1.1.1.37); succinate dehydrogenase, or succinate: (acceptor) oxidoreductase (EC 1.3.99.1); lactate dehydrogenase, or L-lactate: NAD oxidoreductase (EC 1.1.1.27); pyruvate carboxylase, or pyruvate: carbon-dioxide-ligase (ADP) (EC 6.4.1.1); isocitrate dehydrogenase (NADP), or *threo*-D₃-isocitrate: NADP oxidoreductase (decarboxylating) (EC 1.1.1.42).

A detailed description of this method which is based on the digitonin method of Schnaitman *et al.* [11], has been published recently [12].

Determination of Pyruvate Carboxylase-Activity without Fractional Extraction

For determining overall pyruvate carboxylase-activity the livers (and other tissues) were excised from ether-anesthetized rats and placed immediately in liquid nitrogen. The tissue could be stored for several days at -20° without significant loss of pyruvate carboxylase-activity. Extracts were prepared by homogenizing one part of tissue with 9 parts (w/v) of 0.1 M potassium phosphate buffer, pH 7.2, in a close fitting glass homogenizer for 5 min. About 5 ml of the homogenate was exposed to 4 successive 10 sec periods of sonication with a Branson Sonifier, model S 75, adjusted to step 5. During this procedure the homogenate was cooled in a NaCl-ice mixture. The sonicated homogenate was centrifuged at $140,000 \times g$ for 30 min at 0° in a Beckman Spinco Ultracentrifuge. In general 0.05 to 0.1 ml of the clear supernatant, diluted 1:20 with H_2O , was immediately assayed for pyruvate carboxylase-activity.

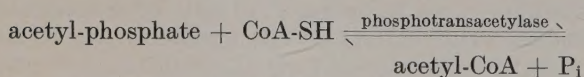
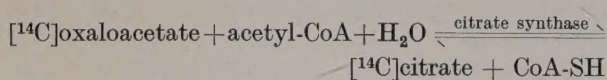
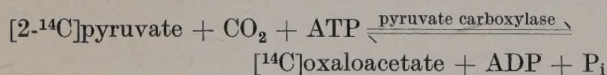
Stability of Pyruvate Carboxylase

When kept in an ice bath, pyruvate carboxylase-activity in the extracts dropped to about 40% within 4 hours. In the presence of 50% glycerol there was no loss in enzyme activity over a period of 10 hours.

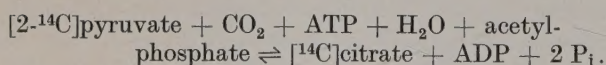
Enzyme Activity Determinations

The activities of adenylate kinase, citrate synthase, fumarase, glutamate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase, isocitrate dehydrogenase and of succinate cytochrome *c* reductase were determined in optical tests which were recently described in detail [12]. The activity of lactate dehydrogenase was measured according to [13].

Pyruvate carboxylase activity was determined in the following system:



i. e. with the overall reaction:



The labelled citrate formed according to the overall reaction is precipitated as silver salt and

counted for radioactivity. The assay procedure as originally described [5] has been modified to some extent and is carried out as follows:

The reaction mixture contained, in a final volume of 0.55 ml, the following components: 40 μ moles $KHCO_3$, 5.0 μ moles $MgSO_4$, 0.55 μ moles CoA-SH, 2.0 μ moles ATP, 2.8 μ moles lithium acetylphosphate, 1 mg crystalline bovine serum albumin, 10 μ g citrate synthase (crystalline) 5 μ g phosphotransacetylase (crystalline), 1.2 μ moles $[2-^{14}C]$ pyruvate ($= 6 \times 10^5$ counts/min); and pyruvate carboxylase. For each determination a blank was run which contained, in addition, 1 mg avidin (2.15 units, Nutritional Biochemicals Corporation, Ohio, U.S.A.). For calculating pyruvate carboxylase-activity, ^{14}C -incorporation of the blank was subtracted from that obtained in the absence of avidin. All determinations were run in duplicates.

After gassing with 95% O_2 — 5% CO_2 the tubes were stoppered and incubated for 30 min in a water bath at 30° . The reaction was stopped by adding 0.2 ml of 10% citric acid and subsequent boiling for 5 min.

Isolation of $[^{14}C]$ citrate by Silver Precipitation

The samples were brought to pH 6.2–6.4 with 2 N KOH, and 2 ml of the following mixture were added: 5 g $AgNO_3$ is dissolved in about 80 ml H_2O and 1 ml 0.5 M Tris solution. The brownish color disappears after addition of 0.15 ml 2 N HNO_3 . Another 3 ml of the Tris solution are added and the mixture is made up to 100 ml with H_2O . The samples are thoroughly mixed with a glass rod and after adding 4 ml acetone—ethanol (1:1, v/v), are kept in the dark for 10 min. The precipitate is then centrifuged and washed thoroughly three times with 4 ml of the acetone—ethanol mixture, dried at about 80° , and, after dissolving in 1 ml 0.1 N HNO_3 , 1 ml H_2O is added. In order to remove Ag^+ , 0.1 ml 5 N HCl is finally added and, after centrifugation, 0.5 ml of the supernatant is mixed with 10 ml of Brays solution and counted for radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer, model 3314. ^{14}C -incorporation is calculated from the specific radioactivity of the labelled pyruvate and pyruvate carboxylase activity is expressed as μ moles $[^{14}C]$ -pyruvate incorporated into citrate per min.

With this procedure a linear reaction rate up to 3 hours of incubation is observed. There is also linear correlation between ^{14}C -citrate formation and the amount of pyruvate carboxylase assayed over a wide range of protein concentration. Further details on the reliability of the test system have been reported [5].

As already pointed out earlier [5], solutions of $[^{14}C]$ pyruvate are degraded to parapyruvate [14,15] during storage thus giving rise to unfavourable high blank values. Commercially obtained $[^{14}C]$ pyruvate

was therefore adjusted to the required final specific activity and divided into smaller samples sufficient for 1 day's experiment; after lyophilization they were stored up to 4 weeks at -20° .

[^{14}C]pyruvate was purchased from The Radiochemical Centre (Amersham, England). All enzymes and biochemical reagents were products of C. F. Boehringer & Soehne (Mannheim, Germany).

RESULTS

Distribution of Pyruvate Carboxylase in Various Tissues of the Rat

Fig.1 demonstrates the distribution of pyruvate carboxylase in various organs of the rat. The highest activities were found in liver and kidney followed by

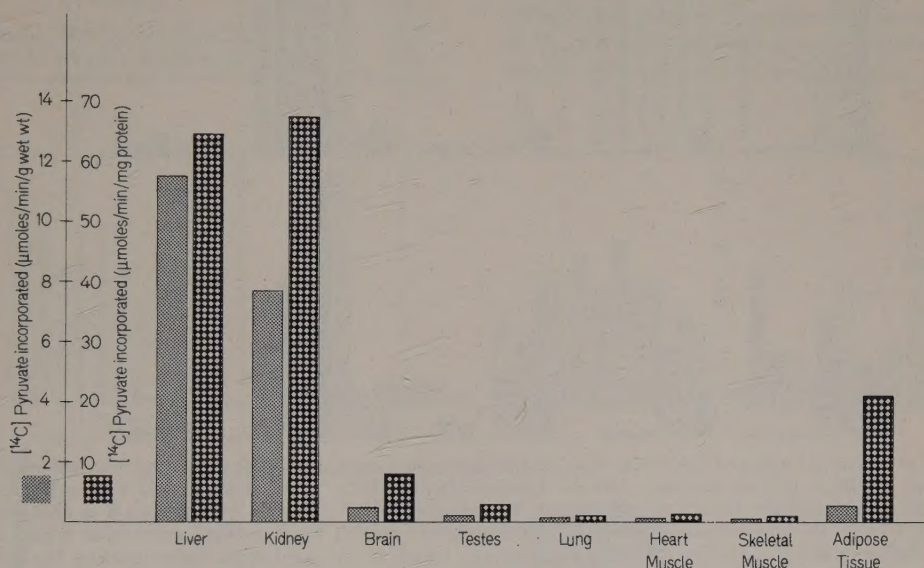


Fig.1. *Distribution of pyruvate carboxylase in various rat tissues.* Mean values of two experiments with fed rats weighing about 190 g. Extraction of the tissues as described for liver

epididymal adipose tissue which contains remarkable amounts of pyruvate carboxylase when calculated on the basis of mg protein. Our data are in good agreement with the findings of Ballard and Hanson [16]. It may be noticed that there is also some pyruvate carboxylase activity in brain. This confirms recent findings of Salganicoff and Koeppe [14]. The other tissues investigated contain only negligible amounts of the enzyme.

Normal Values of Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase Activity

The activities of pyruvate carboxylase and of phosphoenolpyruvate carboxykinase as determined in livers of various species including human are listed in Table 1.

Table 1. *Activity of pyruvate carboxylase and of phosphoenolpyruvate-carboxykinase in the livers of various species*

Values are given in units/g fresh wt., 1 unit corresponding to 1 μmole of substrate converted per min at $30^{\circ} \pm \text{S. D.}$ Human liver samples were obtained from patients during surgery. Rat liver was taken from ether-anesthetized animals

Species	Number of observations	Pyruvate carboxylase	Phosphoenolpyruvate carboxykinase
units/g fresh wt.			
Human	5	1.1 ± 0.7	10.2 ± 2.8
Rat, fed body wt. > 300 g	10	8.7 ± 1.3	2.7 ± 0.5
Rat, fed body wt. 250–300 g	6	11.1 ± 0.9	2.3 ± 0.8
Guinea pig	1	1.1	8.8

Intracellular Localization of Pyruvate Carboxylase

Fig.2 summarizes the distribution of the activities of extra- and intramitochondrial enzymes obtained by fractional extraction of rat liver. The activities of the listed enzymes were measured in 5 different extracts which were prepared by successive steps from the same liver sample. Fractions A, B, and C contain the soluble extramitochondrial enzymes. In Fig.2, lactate dehydrogenase is a representative of this enzyme group. More than 90% of its total cellular activity is found in the extracts A, B, and C. The absolute activities and also the specific activities of lactate dehydrogenase or other extramitochondrial enzymes decrease from fraction A to fraction C. A different extraction pattern is obtained with respect to intramitochondrial enzymes, such as

citrate synthase, glutamic dehydrogenase and 3-hydroxyacyl-dehydrogenase. Only negligible activities of these enzymes can be detected in the two sucrose extracts (A, B) or in the phosphate extract (C) of the minced tissue. 93–98% of the total hepatic activities of these enzymes is found in extract D which is obtained after thorough mechanical disintegration of the tissue. Small amounts of soluble mitochondrial enzymes are finally detected in the resuspended sediment fraction S. A third type of extraction is observed in the case of malate dehydrogenase. It is

fractions D and S which are characterized as mitochondrial extracts due to the high specific activities of the other intramitochondrial enzymes. Thus, by this technique a cytoplasmatic location of pyruvate carboxylase was not observed.

Theoretically, the lack of pyruvate carboxylase activity in the soluble extramitochondrial fraction could be ascribed to the presence of an enzyme inhibitor in these fractions. This was examined by determining pyruvate carboxylase activity in the different fractions either separately or after re-

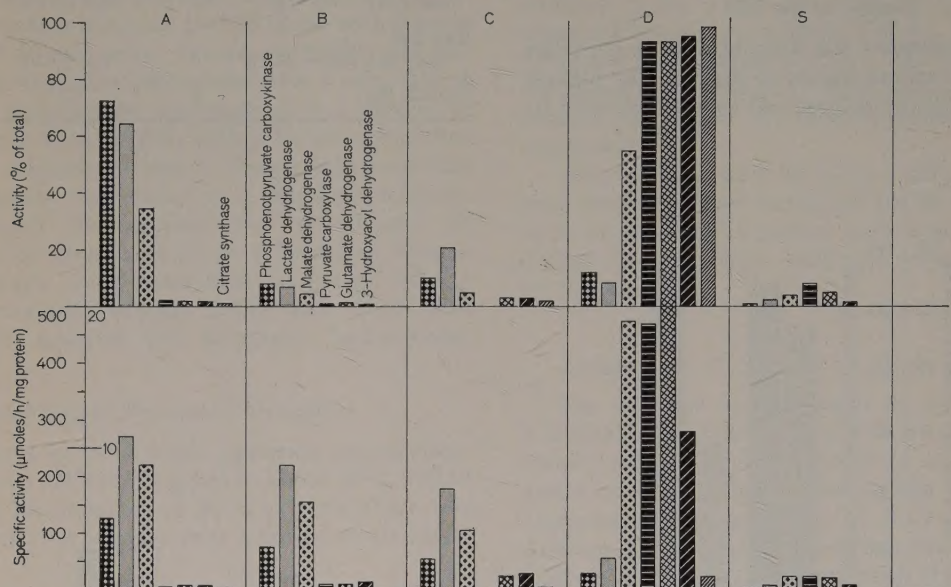


Fig. 2. Fractional extraction of rat liver. The tissue was passed through a special meat grinder and was suspended in a 19-fold volume of sucrose medium (0.3 M sucrose, 10 mM triethanolamine-HCl, 2 mM EDTA, pH 7.2). After 15 min stirring, the suspension was centrifuged for 15 min at $144,000 \times g$. The supernatant is fraction A. Fraction B is obtained by repeating the extraction for 15 min in fresh sucrose medium, and fraction C results from a third extraction which is performed in 0.1 M phosphate buffer, pH 7.2. Fraction D is the supernatant which is obtained after disintegrating the sediment of fraction C in 0.1 M phosphate buffer by 2-min treatment with the Ultra-Turrax (Janke und Kunkel KG., Staufen i. Br., Germany). Fraction S is the resuspended final sediment. Enzyme activities were measured in all fractions and for each fraction the percentage of the total activity and the specific activity were calculated. Because of the great discrepancies of the specific activities there are two different scales: The outer scale (0–500) gives the values for the specific activities of lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase and 3-hydroxyacyl dehydrogenase, while the inner scale (0–20) is that for pyruvate carboxylase, phosphoenolpyruvate carboxylase and citrate synthase. Mean values of four experiments, except phosphoenolpyruvate carboxylase and the specific activity of pyruvate carboxylase, which was determined in one experiment

the well known biphasic solubilization of the extra-mitochondrial and intramitochondrial isoenzymes of malate dehydrogenase [13].

As indicated in Fig. 2, pyruvate carboxylase activity, during fractional extraction, follows exactly that of the intramitochondrial enzymes, such as citrate synthase, glutamic dehydrogenase and 3-hydroxyacyl-dehydrogenase. Less than 2% of the total cellular activity of pyruvate carboxylase can be detected in the extramitochondrial space (fractions A, B, and C). 97% to 98% of the total pyruvate carboxylase activity of the liver is present in the two

combination of the fractions. As shown in Table 2 the sum of the activities measured in the single extracts corresponds very closely to the overall activity as determined in the mixtures. This excludes the presence of an inhibitor of pyruvate carboxylase in the soluble fraction.

Localization of Pyruvate Carboxylase within the Mitochondrion

In a recent communication [12], a method has been reported which allows separate extraction of

Table 2. *Pyruvate carboxylase activities in liver fractions before and after recombination*

After mixing the combined fractions were kept for 60 min at 0°. The classification of the fractions is the same as in Fig. 2

	Fraction	Pyruvate carboxylase activity
		mU
Expt. I	0.5 ml A	0.5
	0.5 ml D	110.1
	Sum A+D, calculated	110.6
	Mixture 0.5 ml A+0.5 ml D	110.6
Expt. II	0.2 ml A	0.2
	0.2 ml B	0.1
	0.2 ml C	0.0
	0.2 ml D	44.0
	0.2 ml S	9.6
	Sum A+B+C+D+S, calculated	53.9
	Mixture A+B+C+D+S, each 0.2 ml	50.2

nant of this preparation contains the soluble citric acid cycle enzymes.

Fig. 3 summarizes our results obtained by this method of fractional extraction of rat liver mitochondria. In the supernatant S 1 which was obtained after centrifugation of digitonin treated mitochondria at $9,500 \times g$, 96% of the total mitochondrial activity of adenylate kinase is found. Washing of the mitochondria in a buffered sucrose medium and centrifugation at $9,500 \times g$ leads to a second supernatant S 2 where only a small amount of adenylate kinase can be detected. Only traces of adenylate kinase are present in the $144,000 \times g$ supernatant S 3 after sonication of the mitochondria suspended in 0.1 M phosphate buffer, pH 7.3. No adenylate kinase activity is detectable in the resulting sediment. A reverse type of extractability is characteristic for pyruvate carboxylase. Under the same experimental conditions, 71% of the total activity of pyruvate carboxylase can be extracted after mechanical distintegration of

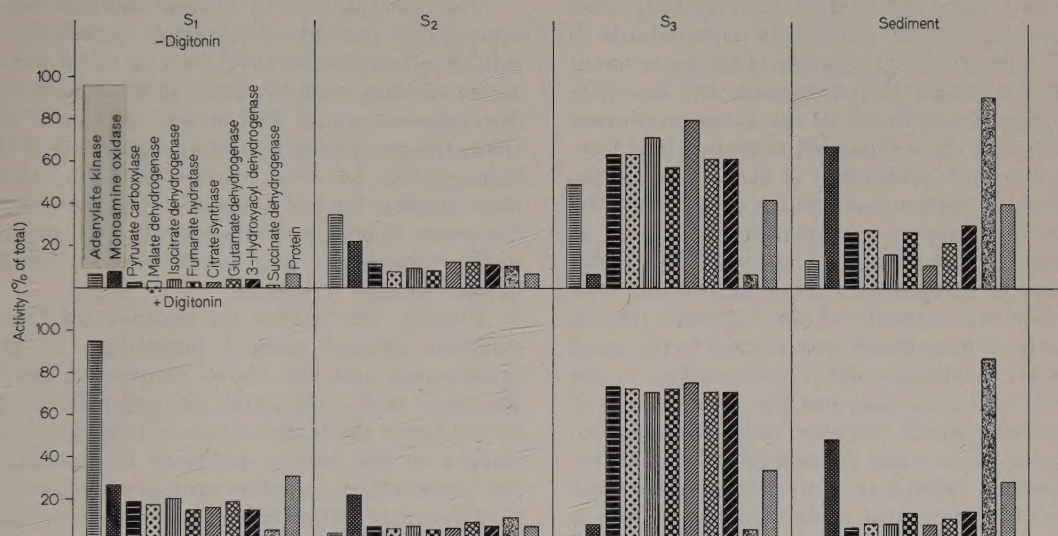


Fig. 3. *Fractional extraction of isolated rat liver mitochondria.* Washed mitochondria are suspended in sucrose medium (0.44 M sucrose, 10 mM triethanolamine-HCl, 2 mM EDTA, pH 7.4) to a concentration of 20–30 mg mitochondrial protein per ml of suspension. 2.1 mg of digitonin are added to 10 mg of mitochondrial protein, and the suspension is incubated for 20 min at +4°. After 10-min centrifugation at $9,500 \times g$ the supernatant S 1 is obtained. The sediment is resuspended in the sucrose medium and centrifuged again for 10 min at $9,500 \times g$. The supernatant is S 2. The sediment is suspended in 0.1 M phosphate buffer, pH 7.2 and exposed for 4×10 sec to sonication in a Branson Sonifier. After 15 min centrifugation at $144,000 \times g$ supernatant 3 (S 3) is obtained. The sediment of this centrifugation is resuspended. For each fraction, the enzyme activities are calculated as percentage of the total activities. Control: mitochondria, identical treatment but without digitonin. Mean values of three experiments

two mitochondrial compartments. By treating mitochondria isolated from rat liver with low concentrations of digitonin [11, 18], the outer mitochondrial membrane is ruptured. Practically 100% of the mitochondrial adenylate kinase is found in the supernatants of such preparations. A second mitochondrial compartment is opened when the digitonin treated mitochondria are exposed to sonication. The super-

the inner mitochondrial membrane. This type of extractability corresponds closely with that of the listed soluble citric acid cycle enzymes [malate dehydrogenase, isocitrate dehydrogenase (NADP), fumarase and citrate synthase]. From the data in Fig. 3 one might conclude that about one fourth of the total activity of pyruvate carboxylase is located within the mitochondrial compartment which is

opened by digitonin treatment. One could arrive at the same conclusion with respect to the soluble citric cycle enzymes which were also present in the supernatants S_1 and S_2 to about 20%.

The primary effect of the digitonin treatment is a rupture of the outer mitochondrial membrane. However, the detachment of the outer mitochondrial membrane also increase the mechanical fragility of mitochondria. In consequence, a small portion of mitochondria is already fragmented completely during the incubation with digitonin and the successive centrifugations. Since fragmented mitochondrial membranes do not sediment at $9,500 \times g$ as do intact mitochondria, it is evident that soluble and structure bound enzymes of the matrix space can be detected in minor quantities in supernatants S_1 and S_2 . Detection of small quantities of succinate dehydrogenase, a marker enzyme of the inner mitochondrial membrane, clearly indicates the presence of fragmented inner mitochondrial membranes in supernatants S_1 and S_2 . Soluble enzymes which are extracted with the bulk of their activity only after sonification, can also be detected in supernatants S_1 and S_2 . With regard to the activity of the inner membrane bound succinate dehydrogenase, the activities of these enzymes are found in the same proportion in S_1 and S_2 as in the sedimented mitochondrial fraction. This constant proportion is also found in the case of pyruvate carboxylase, which proves that the activity of this enzyme in supernatants S_1 and S_2 results from a small portion of mitochondria which are fragmented completely during incubation with digitonin. Yet the majority of the digitonin treated mitochondria remains intact with respect to the inner mitochondrial membrane and, corresponding to the soluble citric acid cycle enzymes, the main portion of pyruvate carboxylase is extracted only after mechanical disruption of the inner mitochondrial membrane.

The identical behaviour of pyruvate carboxylase and the soluble citric acid cycle enzymes suggest a location within the same compartment of mitochondria. This compartment is different from a second mitochondrial space which is opened completely by digitonin treatment and from which the total activity of adenylate kinase can be extracted. From a comparison of enzymological data with electron microscopical findings [12, 19–21] it is suggested that the site of adenylate kinase in liver mitochondria corresponds to the cristae space and that the citric acid cycle enzymes as well as pyruvate carboxylase are located within the matrix space.

DISCUSSION

Our data reported here seem hardly compatible with the view of a "soluble", extramitochondrial pyruvate carboxylase in rat liver. Almost 100% of pyruvate carboxylase activity could be recovered in

the particulate fraction together with the other enzymes of mitochondrial origin such as glutamic dehydrogenase, citrate synthase and hydroxyacyl dehydrogenase. Our data do not completely exclude the possibility that some of the pyruvate carboxylase activity is extracted from other structural elements than mitochondria such as lysosomes, endoplasmic reticulum, nuclei, etc. This seems rather unlikely in view of the evidence from the literature that liver microsomes contain only negligible—if any pyruvate-carboxylase activity [1, 7]. The pyruvate carboxylase activity reported to accompany the nuclear fraction [7] may not truly have been of nuclear origin since the fractionation procedure employed in this study is far from yielding pure nuclei. Therefore contamination by mitochondria and by incompletely broken cells cannot be excluded as the source of pyruvate carboxylase in the "nuclear" fraction described. Whether lysosomes contain pyruvate carboxylase is not known although this possibility seems rather unlikely.

The observations by several authors who found appreciable amounts of pyruvate carboxylase in the soluble extramitochondrial fraction of rat liver [6–8] are at variance with the findings reported here. These discrepancies would find an explanation if one considers the possibility that the preparation of the liver homogenates has been carried out less carefully in their studies. Partial damage to the mitochondria in the course of homogenisation could for instance result in the appearance of pyruvate carboxylase activity in the "soluble" fraction.

Finally, our studies on isolated rat liver mitochondria showed marked parallelism of pyruvate carboxylase and the above mentioned enzymes of the citric acid cycle which are generally believed to be located in the matrix space of mitochondria. With respect to the carbon pathway in gluconeogenesis our observations therefore strongly support the view that pyruvate, before being converted to oxaloacetate, has to enter the mitochondrion. Oxaloacetate so generated within the mitochondrion would thus be able to participate in the well known dicarboxylic acid shuttle as proposed by several authors [22–24].

The skilful technical assistance of Mrs. Gertraud Lusch, Mrs. Eva Prüsse-Evertz and Miss Barbara Stukowski is greatly acknowledged. We are also much indebted to Dr. Max Alexander Schmid, head of the surgical department of the Schwabing City Hospital, Munich, and to Dr. K. Schultis, surgical department, Univ. of Giessen, for providing the specimens of human liver for our enzyme studies.

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REFERENCES

1. Keech, D. B., and Utter, M. F., *J. Biol. Chem.* 238 (1962) 2609.
2. Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T., and Yoshida, T., *Biochem. J.* 86 (1963) 22.

3. Lardy, H. A., Paetkau, V., and Walter, P., *Proc. Natl. Acad. Sci. U. S.* 53 (1965) 1410.
 4. Freedman, A. D., and Kohn, L. D., *Intern. Congr. Biochem.*, 6th, New York 1964, Abstr. IX-23.
 5. Struck, E., Ashmore, J., and Wieland, O., *Enzymol. Biol. Clin.* 7 (1966) 38.
 6. Henning, H. V., Stumpf, B., Ohly, B., and Seubert, W., *Biochem. J.* 344 (1966) 274.
 7. Ballard, F. J., and Hanson, R. W., *Biochem. J.* 104 (1967) 866.
 8. Bargon, N., Grillo, M. A., Rinaudo, M. T., and Fossa, T., *Hoppe-Seyler's Z. Physiol. Chem.* 349 (1968) 275.
 9. Goebell, H., and Pette, D., *Enzymol. Biol. Clin.* 8 (1967) 161.
 10. Pette, D., In *Praktische Enzymologie* (edited by F. Schmidt), Huber Verlag, Bern 1968, p. 15.
 11. Schnaitman, C., Erwin, V., and Greenawalt, I., *J. Cell. Biol.* 32 (1967) 719.
 12. Brdiczka, D., Pette, D., Brunner, G., and Miller, F., *European J. Biochem.* 5 (1968) 294.
 13. Delbrück, A., Zebe, E., and Bücher, T., *Biochem. Z.* 331 (1959) 273.
 14. Korff, R. W. v., *Anal. Biochem.* 8 (1964) 171.
 15. Silverstein, E., and Boyer, P. D., *Anal. Biochem.* 8 (1964) 470.
 16. Ballard, F. J., and Hanson, R. W., *J. Lipid Res.* 8 (1967) 73.
 17. Wieland, O., unpublished data.
 18. Levy, M., Toury, R., and Andre, I., *Compt. Rend. Ser. D.*, 262 (1966) 1595.
 19. Sottocasa, G. L., Kuylenstierna, B., Ernster, L., and Bergstrand, A., *J. Cell Biol.* 32 (1967) 415.
 20. Sottocasa, G. L., Kuylenstierna, B., Ernster, L., and Bergstrand, A., In *Methods in Enzymology* (edited by S. P. Colowick, and N. O. Kaplan), Academic Press, New York 1967, Vol. X, p. 448.
 21. Parsons, D. F., Williams, G. R., Thompson, W., Wilson, D., and Chance, B., In *Mitochondrial Structure and Compartmentation* (edited by E. Quagliariello, S. Papa, E. C. Slater, and I. M. Tager), Adriatica Editrice, Bari 1967, p. 29.
 22. Lardy, H. A., *Harvey Lectures*, 60 (1966) 261.
 23. Struck, E., Ashmore, J., and Wieland, O., *Advan. Enzyme Regulation*, 4 (1966) 219.
 24. Walter, P., Paetkau, V., and Lardy, H. A., *J. Biol. Chem.* 241 (1966) 2523.
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Die vorliegende Arbeit entstand im wesentlichen während der Medizinalassistentenzeit 1967—68.

